Do histological, immunohistochemical, and metabolic (radioiodine and fluorodeoxyglucose uptakes) patterns of metastatic thyroid cancer correlate with patient outcome?

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Abstract

The aim of this study is to search for relationships between histology, radioiodine (¹³¹I) uptake, fluorodeoxyglucose (FDG) uptake, and disease outcome in patients with metastatic thyroid cancer. Eighty patients with metastatic thyroid cancer (34 males, 46 females, mean age at the time of the diagnosis of metastases: 55 years) were retrospectively studied. All patients were treated with radioactive iodine and evaluated by FDG-positron emission tomography (PET). Primary tumor tissue sample was available in all cases. Forty-five patients (56%) had a papillary, 12 (15%) a follicular, and 23 (29%) a poorly differentiated thyroid cancer. Cellular atypias, necrosis, mitoses, thyroid capsule infiltration, and vascular invasion were frequently detected (70, 44, 52, 60, and 71% respectively). Metastases disclosed FDG uptake in 58 patients (72%) and ¹³¹I uptake in 37 patients (45%). FDG uptake was the only significant prognostic factor for survival ($P=0.02$). The maximum standardized uptake value and the number of FDG avid lesions were also related to prognosis ($P=0.03$ and 0.009). Age at the time of the diagnosis of metastases ($P=0.001$) and the presence of necrosis ($P=0.002$) were independent predictive factors of FDG uptake. Radioiodine uptake was prognostic for stable disease ($P=0.001$) and necrosis for progressive disease at 1 year ($P=0.001$). Histological subtype was not correlated with in vivo tumor metabolism and prognosis. In conclusion, FDG uptake in metastatic thyroid cancer is highly prognostic for survival. Histological subtype alone does not correlate with ¹³¹I/FDG uptake pattern and patient outcome. Well-differentiated thyroid cancer presenting histological features such as necrosis and FDG uptake on PET scan should be considered aggressive differentiated cancers.

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Introduction

Distant metastases occur in <10% of patients with non-anaplastic follicular cell-derived thyroid cancer. Radioiodine is the most frequently used systemic treatment, and may be associated with local treatment modalities (Robbins & Schlumberger 2005).

In metastatic patients, 10-year survival rates ranging from 25 to 42% were reported. It is less favorable in older patients, in those with poorly differentiated cancer, multiple and large metastases, and in the absence of radioiodine uptake (Schlumberger 1998, Durante et al. 2006). Furthermore, fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET) scan in the metastases is associated with poor survival and poor response to radioiodine treatment (Wang et al. 2000, 2001, Robbins et al. 2006). In fact, cancer cells show an increased metabolism of glucose and a high-FDG uptake, but FDG uptake is higher in
fast-growing and more aggressive tumors, and among differentiated thyroid tumors well-differentiated ones retain less FDG, whereas the more aggressive ones appear to have a higher FDG uptake (Feine et al. 1996).

Nonanaplastic follicular cell-derived thyroid cancers are classified as well-differentiated (papillary and follicular) or poorly differentiated carcinoma, a classification that has a prognostic impact on survival (Volante et al. 2004a,b). As already shown in other tumor types, recent studies in thyroid cancer have demonstrated the prognostic significance of necrosis and proliferation, estimated by the number of mitoses (Akslen & LiVolsi 2000, Hiltzik et al. 2006). Furthermore, immunohistochemistry may detect the expression of proteins involved in iodine metabolism (sodium iodine symporter (NIS), thyroglobulin (Tg), thyroid peroxidase (TPO), apical iodine transporter (AIT), and dual-oxidase (p138-DUOX)), FDG uptake (glucose transporter 1 (GLUT-1) and hexokinase; Dupuy et al. 2000, De Deken et al. 2002, Schönberger et al. 2002, Lacroix et al. 2004, Schlumberger et al. 2007), and angiogenesis, such as vascular endothelial growth factor (VEGF) and VEGF receptor (VEGF-R) (Bunone et al. 1999, Katoh et al. 1999, Klein et al. 2001, Lennard et al. 2001). These three features were shown to be related to thyroid cancer prognosis. Finally, genetic abnormalities including BRAF and p53 mutations are also involved in tumor aggressiveness and dedifferentiation (Fagin et al. 1993, Nikiforova et al. 2003, Xing 2005, Fugazzola et al. 2006, Durante et al. 2007). However, the independent prognostic significance of these multiple parameters remains unknown.

The aim of this study is to establish an imaging, histological, immunohistochemical, and mutational profile to identify prognostic parameters in metastatic patients with nonanaplastic follicular cell-derived thyroid cancer.

Patients and methods

Patients

Among the 95 patients treated and followed up at IGR for distant metastases from follicular cell-derived thyroid cancer between 2002 and 2007, 80 patients were retrospectively selected, according to the following criteria: i) available primary tumor samples for histological review; ii) absence of undifferentiated component; iii) at least one treatment with radioactive iodine $^{131}$I to assess the presence of radioiodine uptake; and iv) FDG-PET scan study at the time of the diagnosis of distant metastases or during follow-up to assess the FDG uptake in the metastases.

In vivo characterization

Metastatic status was defined by the presence of distant lesions by radioiodine whole body scan (WBS) or computed tomography (CT) and/or magnetic resonance imaging (MRI).

Radioiodine WBS was performed 3–5 days after the administration of a standard activity of $^{131}$I (3700 MBq–100 mCi) following withdrawal of thyroid hormone treatment during 4 weeks (TSH > 30 mU/l). Anterior and posterior images were obtained using a dual-head gamma camera equipped with high-energy collimators and thick crystals (AXIS, General Electric Medical System, Milwaukee, WI, USA).

FDG-PET scan was performed using a Biograph LSO PET/CT (Siemens Medical System, Malvern, PA, USA). Image acquisition was obtained 60 min after the injection of 4–6 MBq/kg of FDG in patients fasting for at least 6 h and with glycaemia <10 mmol/l. FDG-PET scan was performed during levothyroxine (L-T$_4$) suppressive treatment (TSH < 0.01 mU/l) in 59 patients, following the withdrawal of thyroid hormone (TSH > 30 mU/l) in 17 patients and after i.m. injection of rhTSH in 4 patients. A CT scan scout was first performed (80 mAs; 110 kV, slice: 5 mm, pitch: 1.5). PET images were then recorded from the skull to the mid-thighs in 3D mode, 4 min per bed position and reconstructed by an iterative method. FDG-PET/CT was interpreted into three planes (axial, coronal, and sagittal) and the maximum standard uptake value (SUVmax) of the most avid lesion was considered for analysis. In each patient, the first FDG-PET scan performed was considered as the reference examination.

Radioiodine and FDG-PET/CT images were both interpreted blindly by two nuclear medicine physicians (D D and S L). First, a visual analysis was performed to detect pathological foci of uptake. A consensus was obtained to classify patients into four groups on the basis of the presence of FDG and/or radioiodine uptake: 1) $^{131}$I+/FDG−, 2) $^{131}$I−/FDG+, 3) $^{131}$I+/FDG+, 4) $^{131}$I−/FDG−. Patients were then classified either as having lung metastases, bone metastases, association of lung and bone lesions or as having metastases in other sites. Lung lesions were divided into micrometastases (<1 cm) and macrometastases (>1 cm) on the basis of their size by CT scan, while bone lesions were divided into single or multiple localizations, as previously reported (Durante et al. 2006). The median interval of time between the diagnosis of metastatic disease and FDG-PET scan evaluation was 1 year (range: 1 month–10 years). In 63 (79%) patients, radioiodine and FDG-PET scans were performed within 1 year and in the remaining 17 (21%)
within an interval of time > 1 year (range: 2–10 years). Serum Tg levels were obtained at the time of metastases diagnosis using the Access kit (Beckman Coulter, Fullerton, CA, USA) and were available during L-T4 treatment in 66 (83%) patients and following thyroid hormone withdrawal in 71 (89%) patients. Anti-Tg antibodies were screened in all sera.

Histological, immunohistochemical, and BRAF mutation analysis

Histology
The 80 histological specimens were reviewed by two experts in thyroid pathology (A A L and B C). Tumors were classified according to the WHO recommendations as papillary (classical or variant), follicular (minimally or widely invasive), and poorly differentiated thyroid cancer (Hedinger et al. 1989). The presence of tumor encapsulation, tumor capsule invasion, thyroid capsule invasion and extension beyond the thyroid capsule, vascular invasion, multifocality, cellular atypia, necrosis (focal or diffuse), and mitoses (count for 2 mm²) were analyzed.

Immunohistochemistry
Paraffin blocks were available for 52 patients. The expression of NIS, AIT, Tg, TPO, p138-DUOX, and p53 was evaluated by immunohistochemistry using specific monoclonal or polyclonal antibodies, as previously described (Lacroix et al. 2004, Faggiano et al. 2007, Pulcrano et al. 2007). For GLUT-1 expression, a rabbit polyclonal antibody was used (A3536; dilution 1/200; incubation time: 60 min, Dako, Glostrup, Denmark). Positive controls for NIS, AIT, Tg, TPO, p138-DUOX, and p53 were normal thyroid tissue and for GLUT-1 the red blood cells (internal controls). Positivity, negativity, percent of positive cells, staining intensity (low, medium, and high), and site (cytoplasm, membrane, and nucleus) were analyzed in each specimen.

BRAF gene sequencing
DNA was extracted from paraffined tumor tissues using DNeasy Extraction kit (Qiagen). A mutation hot spot region located in exon 15 of BRAF gene was analyzed after PCR. Sequencing reactions were performed using BigDye Terminator Cycle Sequencing kit and analyzed by 48-capillary 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Each experiment was performed independently two times. Sequence reading and alignment were performed from the exonic part using SeqScape software (Applied Biosystems). The same primers were used for PCR (forward primer 5’-CCTTACTACACCTCAGAT-3’; reverse primer 5’-GACCGACTCCATCGAGATTT-3’).

Follow-up
Patients with radioiodine uptake in metastatic lesions were treated every 6–12 months with a standard activity of radioiodine (3700 MBq–100 mCi) following L-T4 withdrawal (median number of treatments: 4, range: 2–8). The PET scan, thorax CT scan, and/or MRI were performed at least once a year in patients with FDG uptake.

Progression-free survival at 1 year after the diagnosis of distant metastases was assessed according to RECIST criteria by CT scan and EORTC criteria by FDG-PET images (Young et al. 1999, Eisenhauer et al. 2009).

Statistical analysis
Statistical analysis was performed using SAS version 9.1.3. System (Cary, NC, USA). The results were evaluated as mean±s.d., median, and range. The evaluation of relative risk associated with FDG and 131I uptake was calculated for each clinical (age at metastases diagnosis, T (tumor) N (lymph nodes) M (metastases) classification (TNM), and metastases size and location), histological (primary tumor histology, necrosis, mitosis, cellular atypia, vascular emboli, and thyroid capsule infiltration), and immunohistological (NIS, Tg, AIT, TPO, GLUT 1, p53, and p138-DUOX staining) variable. A relationship between these variables, in vivo metabolic pattern (131I and FDG uptakes), and overall survival/progression-free survival was evaluated by a $\chi^2$ test (univariate analysis). $P$ value <0.05 was considered statistically significant. Statistically significant variables were analyzed in the multivariate analysis by logistic regression model except for the SUVmax value and the number of FDG avid lesion. Overall survival and progression-free survival at 1 year were estimated from the date of the first PET scan by the Kaplan–Meier method.

Results
Clinical characteristics
Eighty patients (34 males, 46 females; mean age at the time of distant metastases diagnosis: 55±19 years) were included. All patients underwent total thyroidectomy and 54 (67%) had a lymph node dissection. Mean tumor size was 36±25 mm (median size: 30 mm). Primary tumor was classified as pT0 in 8 (10%) cases, pT1 in 8 (10%) cases, pT2 in 13 (16%)
cases, pT3 in 24 (30%) cases, and pT4 in 27 (34%) cases. Twenty-six (32%) patients were Nx, 14 (18%) were N0, and 40 (50%) were N1. Distant metastases were present initially in 21 patients and in the other 59 patients were discovered at a mean time of 2.4 ± 4.7 years after the diagnosis of the primary tumor. Metastases were located in the lungs only in 43 (54%) patients, in the bones only in 16 (20%), in the lungs and bones in 15 (19%), and in other sites in 6 (7%) patients (liver in 4, brain in 1, and muscle in 1). At the time of the diagnosis of metastases, among patients with lung lesions, 22 had macrometastases (lesions >1 cm in diameter) and 36 had micrometastases (<1 cm). Among patients with bone metastases, 21 had multiple lesions and the remaining 10 had a single lesion.

At the time of the diagnosis of distant metastases, the serum Tg levels during L-T4 treatment were undetectable in 8 patients, ranging from 1 to 20 ng/ml in 23 patients, from 21 to 100 ng/ml in 8 patients, and were above 100 ng/ml in 27 patients (median level: 16 ng/ml, range: 0–33,506 ng/ml). The serum Tg levels following L-T4 withdrawal were undetectable in 6 patients, ranging from 1 to 100 ng/ml in 26 patients, from 101 to 1000 ng/ml in 15 patients, and were above 1000 ng/ml in 24 patients (median level: 209 ng/ml, range: 0–61,700 ng/ml). The six patients with undetectable serum Tg had no detectable serum anti-Tg antibodies and all had a poorly differentiated thyroid carcinoma. In the remaining 17 patients with a poorly differentiated carcinoma, the serum Tg level ranged from 3.3 to 20,300 ng/ml during L-T4 treatment and from 20 to 61,700 ng/ml following L-T4 withdrawal.

Patients were followed up for a mean of 4.2 ± 4.3 years after the diagnosis of distant metastases.

### Histology

Forty-five patients (56%) had a papillary cancer (65% classical, 22% tall cells, 7% follicular variant, 2% columnar cells, 2% oncocytic cells, and 2% diffuse sclerosing variant), 12 (15%) had a widely invasive follicular cancer (33% with oncocytic cells), and 23 (29%) had a poorly differentiated cancer (48% with oncocytic cells). Patients with papillary thyroid cancer presented more frequently metastases in the lungs (P=0.01) and patients with follicular carcinoma in the bones (P=0.01).

Cellular atypias and the presence of necrosis and mitosis were significantly more frequently observed in the poorly differentiated histotype, but also were present in some papillary and follicular carcinomas (Table 1). Extension beyond the thyroid capsule was detected in 48 (60%) tumors and vascular invasion in 56 (71%), without any relationship with the histological subtype (P=0.9).

### Radioiodine and FDG uptake

Eighteen (23%) patients were 131I+/FDG−, 39 (49%) were 131I−/FDG+ , 19 (24%) were 131I+/FDG+, and 4 were (5%) 131I−/FDG−. A total of 58 (72%) patients had FDG uptake on PET scan and 37 had 131I uptake (45%).

Twenty-two patients (28%) had no FDG avid lesions, 24 (30%) had 1–5 FDG avid lesions, 17 (21%) had 6–10 avid lesions, and 17 (21%) had more than 10 FDG avid lesions. SUVmax in the most avid lesion was <5 in 40 patients (50%) including the 22 patients with no FDG uptake, between 5 and 10 in 14 (18%), and higher than 10 in 26 (32%) patients. The median SUVmax of the hottest lesion in patients who underwent FDG-PET after the L-T4 withdrawal or rhTSH was 9.7 (range: 1.2–35) compared to 10.9 (range: 1.3–50) in those who underwent FDG/PET under L-T4 suppression. In the univariate analysis, FDG uptake was correlated to age >45 years (P=0.001), lung macrometastases (P=0.02), tumor differentiation (P=0.01), cellular atypias (P=0.001), tumor necrosis (P=0.01), and mitoses (P=0.008). Of note, 6 among the 14 patients with lung metastases without FDG uptake presented only small lesions of <5 mm. In the multivariate analysis, FDG uptake was independently correlated with the age at the time of the diagnosis of metastases (P=0.001) and the presence of necrosis (P=0.002; Tables 2 and 3). In the subgroup of 57 patients with well-differentiated cancer, the multivariate analysis showed that only the age at the diagnosis of metastases (P=0.02) but not necrosis was correlated with FDG uptake (Table 3).

<table>
<thead>
<tr>
<th>Histology</th>
<th>Cellular atypias (P=0.01)</th>
<th>Necrosis (P=0.01)</th>
<th>Mitoses (P=0.02)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTC</td>
<td>10/11a (90%)</td>
<td>3/11a (27%)</td>
<td>5/11a (45%)</td>
</tr>
<tr>
<td>PTC</td>
<td>23/45 (51%)</td>
<td>13/45 (29%)</td>
<td>15/45 (33%)</td>
</tr>
<tr>
<td>PDTC</td>
<td>21/21b (100%)</td>
<td>18/22b (81%)</td>
<td>21/23 (93%)</td>
</tr>
<tr>
<td>Total</td>
<td>54/77 (70%)</td>
<td>34/78 (44%)</td>
<td>41/79 (52%)</td>
</tr>
</tbody>
</table>

FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; PDTC, poorly differentiated thyroid cancer.
a1 missing.  
b2 missing.
The number of FDG avid lesions and SUVmax value were not related to the histological type ($P=0.4$ and $0.8$ respectively).

Radioiodine uptake was present in $37$ patients ($45\%$). In the univariate analysis, radioiodine uptake was correlated with female gender ($P=0.03$) and tumor differentiation ($P=0.01$). In the multivariate analysis, female gender resulted as the only independent predictive factor ($P=0.01$).

### Overall survival

Fourteen patients died at a mean time of $3 \pm 3.7$ years (median: $2$ years) after the diagnosis of metastases. Median age at death was 64 years (range: 45–83 years). Ten of these patients had a papillary carcinoma, one had a follicular carcinoma, and three had a poorly differentiated carcinoma. All these 14 patients were FDG-PET positive (4 were $^{131}$I-/FDG+ and 10 were $^{131}$I+/FDG+). FDG uptake was the only significant prognostic factor for overall survival ($P=0.02$; Table 4). In particular, overall 2-year survival was $60\%$ for PET-positive and $100\%$ for PET-negative patients, with no difference between the $^{131}$I-/FDG+ and $^{131}$I+/FDG+ patients ($P=0.2$; Fig. 1A and B). Furthermore, the intensity of FDG uptake and the number of

<table>
<thead>
<tr>
<th>Factor</th>
<th>Number of patients at risk</th>
<th>%</th>
<th>Relative risk (95% CI)</th>
<th>$P$ value (UNIV)</th>
<th>$P$ value (MULTIV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, &gt;45 years/≤45 years</td>
<td>48/60 vs 10/20</td>
<td>80/50</td>
<td>4.0 (1.40–11.80)</td>
<td>$&lt;10^{-3}$</td>
<td>$&lt;10^{-3}$</td>
</tr>
<tr>
<td>Gender, females/males</td>
<td>30/46 vs 28/34</td>
<td>65/82</td>
<td>0.40 (0.14–1.16)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Metastases site</td>
<td></td>
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<tr>
<td>Bone/lung</td>
<td>11/16 vs 31/43</td>
<td>69/72</td>
<td>0.85 (0.24–2.97)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Bone + lung/lung</td>
<td>12/15 vs 31/43</td>
<td>80/72</td>
<td>1.55 (0.37–6.50)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Others/lung</td>
<td>4/6 vs 31/43</td>
<td>67/72</td>
<td>0.67 (0.24–2.97)</td>
<td>NS</td>
<td></td>
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<tr>
<td>Lung metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Micro/macro</td>
<td>22/36 vs 20/22</td>
<td>61/90</td>
<td>6.4 (1.30–31.50)</td>
<td>NS</td>
<td>0.02</td>
</tr>
<tr>
<td>Bone metastases</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Single/multiple</td>
<td>9/10 vs 15/21</td>
<td>90/71</td>
<td>4.2 (0.40–39.0)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>TNM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tx/T1</td>
<td>4/8 vs 6/8</td>
<td>75/50</td>
<td>2.24 (0.25–20.10)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Tx/T2</td>
<td>4/8 vs 10/13</td>
<td>77/50</td>
<td>2.5 (0.35–10.18)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Tx/T3</td>
<td>4/8 vs 16/25</td>
<td>64/50</td>
<td>1.37 (0.24–7.32)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Tx/T4</td>
<td>4/8 vs 22/27</td>
<td>81/50</td>
<td>3.29 (0.55–19.69)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Nx/N0</td>
<td>19/27 vs 11/14</td>
<td>70/78</td>
<td>1.35 (0.29–2.32)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC/PTC</td>
<td>6/12 vs 31/45</td>
<td>50/69</td>
<td>0.45 (0.12–1.65)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PDTC/PTC</td>
<td>21/23 vs 31/45</td>
<td>91/69</td>
<td>4.76 (0.97–23)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Necrosis, present/absent</td>
<td>30/34 vs 28/44</td>
<td>88/64</td>
<td>4.29 (1.36–11.79)</td>
<td>$&lt;10^{-2}$</td>
<td>$&lt;0.002$</td>
</tr>
<tr>
<td>Mitosis, present/absent</td>
<td>35/41 vs 23/39</td>
<td>85/59</td>
<td>4.87 (1.03–23.1)</td>
<td>0.008</td>
<td>NS</td>
</tr>
<tr>
<td>Cellular atypias, present/absent</td>
<td>45/53 vs 12/24</td>
<td>83/50</td>
<td>5.62 (1.88–16.87)</td>
<td>$&lt;10^{-3}$</td>
<td>NS</td>
</tr>
<tr>
<td>Vascular invasion, present/absent</td>
<td>42/58 vs 9/12</td>
<td>72 vs 75</td>
<td>0.87 (0.21–3.65)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Extension beyond thyroid capsule, present/absent</td>
<td>34/48 vs 18/23</td>
<td>71 vs 78</td>
<td>0.67 (0.21–2.17)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

UNIV, univariate analysis; MULTIV, multivariate analysis; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer; CI, confidence interval.

The number of FDG avid lesions and SUVmax value were not related to the histological type ($P=0.4$ and $0.8$ respectively).

Radioiodine uptake was present in $37$ patients ($45\%$). In the univariate analysis, radioiodine uptake was correlated with female gender ($P=0.03$) and tumor differentiation ($P=0.01$). In the multivariate analysis, female gender resulted as the only independent predictive factor ($P=0.01$).
lesions with FDG uptake were prognostic for survival. Overall 2-year survival was 85% for patients with an SUVmax <5 and 55% for patients with an SUVmax higher than 10 \((P=0.03; \text{Fig. 1C})\). Two-year survival was 100% for patients without lesions with FDG uptake, between 70 and 80% for patients with 1–10 lesions, and 50% for patients with more than 10 lesions with FDG uptake \((P=0.009; \text{Fig. 1D})\). In the subgroup of 57 patients with a well-differentiated cancer, overall survival also was related to FDG uptake, SUVmax, and the number of FDG avid lesions \((P=0.001, 0.02, \text{and } 0.02 \text{ respectively})\), and to radioiodine uptake \((P=0.03)\). In these patients, FDG and radioiodine uptakes were two independent prognostic factors for survival \((P=0.001 \text{ and } 0.03)\). In particular, overall 2-year survival was 95% for \(^{131}\text{I}+\) patients compared with 80% for \(^{131}\text{I}−\) patients.

### Progression-free survival

Among the 60 patients who were evaluated for tumor progression at 1 year, 16 (27%) had disease progression. Among the patients with progressive disease, median age was 62 years (range: 21–86), 9 had a papillary carcinoma and 7 had a poorly differentiated carcinoma, 15 were FDG+ (12 were \(^{131}\text{I}−/\text{FDG}+\) and 3 were \(^{131}\text{I}+/\text{FDG}+)\) and only one was \(^{131}\text{I}+/\text{FDG}−\). In the univariate analysis, disease progression was related to the absence of radioiodine uptake

\[
\begin{array}{lcccc}
\text{Factor} & \text{Alive/total} & \% & P \text{ value} \\
\hline
\text{Age, } > 45 \text{ years}/< 45 \text{ years} & 50/60 \text{ vs } 16/20 & 80 \text{ vs } 83 & \text{NS} \\
\text{Gender, females/males} & 37/46 \text{ vs } 29/34 & 80 \text{ vs } 85 & \text{NS} \\
\text{Metastases site} & & & \text{NS} \\
\text{Lung} & 37/43 & 86 & \\
\text{Bone} & 14/16 & 87 & \\
\text{Bone + lung} & 14/15 & 73 & \\
\text{Others} & 4/6 & 67 & \\
\text{Lung metastases} & & & \text{NS} \\
\text{Micro/macro} & 30/50 \text{ vs } 6/11 & 60 \text{ vs } 55 & \\
\text{Bone metastases} & & & \text{NS} \\
\text{Single/multiple} & 8/26 \text{ vs } 2/6 & 31 \text{ vs } 33 & \text{NS} \\
\text{TNM} & & & \text{NS} \\
\text{Tx} & 6/6 & 100 & \\
\text{T1} & 6/8 & 75 & \\
\text{T2} & 11/14 & 79 & \\
\text{T3} & 20/24 & 83 & \\
\text{T4} & 21/26 & 81 & \\
\text{Nx} & 17/24 & 71 & \\
\text{N0} & 12/14 & 86 & \\
\text{N1} & 35/40 & 87 & \\
\text{Histology} & & & \text{NS} \\
\text{PTC} & 35/45 & 78 & \\
\text{FTC} & 11/12 & 92 & \\
\text{PDTC} & 20/23 & 87 & \\
\text{Necrosis, present/absent} & 28/34 \text{ vs } 36/44 & 81 \text{ vs } 82 & \text{NS} \\
\text{Mitosis, present/absent} & 17/21 \text{ vs } 49/59 & 80 \text{ vs } 83 & \text{NS} \\
\text{Cellular atypias, present/absent} & 45/53 \text{ vs } 18/24 & 85 \text{ vs } 75 & \text{NS} \\
\text{Vascular invasion, present/absent} & 48/58 \text{ vs } 10/12 & 83 \text{ vs } 83 & \text{NS} \\
\text{Extension beyond the thyroid capsule, present/absent} & 41/48 \text{ vs } 17/23 & 85 \text{ vs } 74 & \text{NS} \\
\text{FDG uptake, present/absent} & 44/58 \text{ vs } 22/22 & 89 \text{ vs } 100 & 0.02 \\
\text{SUVmax} & & & 0.03 \\
\text{<} 10 & 48/54 & 88 & \\
\text{>} 10 & 18/26 & 69 & \\
\text{Total number of avid FDG lesions} & & & 0.009 \\
\text{<} 10 & 54/63 & 86 & \\
\text{>} 10 & 12/17 & 71 & \\
\text{Radioiodine uptake: present/absent} & 33/37 \text{ vs } 33/43 & 89 \text{ vs } 77 & \text{NS} \\
\end{array}
\]

PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer.
(P = 0.005), the presence of FDG uptake (P = 0.01), SUVmax value (P = 0.02), total number of FDG avid lesions (P = 0.01), and to the presence of necrosis and mitosis (P = 0.001 and 0.03 respectively). In the multivariate analysis, the absence of radioiodine uptake (P = 0.001) and the presence of necrosis (P = 0.001) were the only two independent significant predictive factors (Table 5). The multivariate analysis performed on the 42 evaluative patients with a well-differentiated cancer provided similar results.

Only 4/80 (5%) patients presented a complete response after radioiodine treatment, defined by negative imaging studies, without residual uptake on a posttherapeutic radioiodine WBS. Complete remission was obtained after a median of 3 years after the diagnosis of metastases and after 2–8 (median: 4) radioiodine treatments. All these four patients were 131I+/FDG–.

**Immunohistochemistry**

Among the 52 paraffin blocks analyzed, NIS was detected in 24 cases (46%) with a range of positive cells of 10–90%; positivity being localized in the cytoplasm in 15 cases (63%), in the nucleus in 5 cases (21%), and in the plasmic membrane in 4 cases (16%). TPO was detected in 27 cases (52%), Tg in 49 cases (94%) with a range of positive cells of 20–100%, AIT in 51 cases (98%) with a range of positive cells of 50–100%, and p138-DUOX in 41 cases (79%) with a range of positive cells of 1–90%. TPO expression was significantly correlated with radioiodine uptake (P = 0.02), but NIS staining did not predict 131I uptake (P = 0.4). NIS (P = 0.6) and TPO (P = 0.2) expressions were not correlated with FDG uptake (P = 0.2 and 0.7 respectively) or prognosis (P = 0.2). No correlation was found between p138-DUOX expression and histological subtype (P = 0.8), and between radioiodine/FDG uptake (P = 0.4) and prognosis (P = 0.5).

GLUT-1 expression was detected in 8/52 cases (15%) and with low percentage of positive cells (1–30%); it was positive in 5 papillary, 2 poorly differentiated, and 1 follicular cancer. Among these eight positive cases, six had FDG uptake on PET scan (five were 131I+/FDG+ and one was 131I+/FDG+), but no correlation was found between GLUT-1 expression and SUVmax value (P = 0.7) and prognosis (P = 0.7).

VEGF was highly expressed in all 52 specimens with a range of positive cells between 20 and 100%.

**Figure 1** (A) Overall survival according to FDG and 131I uptakes (P = 0.2). (B) Overall survival according to FDG uptake on PET scan (P = 0.02). (C) Overall survival according to SUVmax value on PET scan (P = 0.03). (D) Overall survival from the PET scan date on the basis of the number of lesions presenting FDG uptake (P = 0.009).
Positive staining for p53 was present in 20/52 tumors and was not correlated with histological type ($P=0.7$), radioiodine/FDG uptake ($P=0.4$), or prognosis ($P=0.7$).

### BRAF mutation

BRAF analysis was performed on 30 tumoral tissue specimens, including 16 papillary, 11 poorly differentiated, and 3 follicular cancers. BRAF mutation at V600 codon was detected in only three tumors, two tall cell variants of papillary cancer, and in one poorly differentiated carcinoma. Cellular atypias were present in the three cases, and necrosis and mitoses in two cases. Tg, AIT, and VEGF were highly expressed and TPO, NIS, and p53 expressions were not detected. P138-DUOX was expressed in two cases and was negative in one case. All these three cases presented lung metastases and were $^{131}$I-FDG+. These three patients were still alive with residual disease at 1, 7, and 8 years after the diagnosis of distant metastases.

### Discussion

Metastatic thyroid cancer represents a heterogeneous disease with different outcomes. In this study, 80 patients with distant metastases from differentiated thyroid carcinoma were retrospectively analyzed. For the first time, a complete in vivo and in vitro metabolic characterization associated with histological and immunohistological analysis was performed in a selected population with distant metastases. The majority of our patients disclosed FDG uptake (72%) and only 45% disclosed radioiodine uptake. This may
be related to a selection bias, primarily due to the referral nature of our institution. Both the availability of FDG-PET/CT and the implementation of molecular-targeted therapy trials in our center favored the referral of patients with aggressive disease. Then, even if most patients underwent the first FDG-PET at the time of metastases diagnosis and the first radiiodine treatment, in some cases FDG-PET was performed later on after the metastases diagnosis in patients who more frequently did not disclose any $^{131}$I uptake. The low remission rate observed following $^{131}$I treatment could also be related to aggressive tumor selection. In this study, the only independent prognostic factor for overall survival in metastatic patients was the presence of FDG uptake in metastatic lesions with a 2-year overall survival rate of 60% for FDG+ patients and 100% for FDG− patients. The FDG uptake level expressed by the SUVmax and the number of avid FDG lesions were also prognostic factors of survival, in accordance with a previous study (Robbins et al. 2006). Our data also confirm that survival was similar in PET-positive patients, either with or without radiiodine uptake ($P=0.2$). It emphasizes the clinical importance of performing FDG-PET in the management of patients with metastatic thyroid cancer. In this study, analyzed patients underwent FDG-PET either under TSH stimulation (after rhTSH injections or after l-T$_4$ withdrawal) or during l-T$_4$ treatment, but this did not change the metastatic and metabolic status (FDG+/FDG−) of our patients and it did not influence the prognostic value of FDG uptake itself, in accordance with a previous study (Robbins et al. 2006). Neither histological subtype nor any immunohistological characteristics influenced the overall survival. This may be due to the low number of samples and of observed deaths or the aggressiveness of differentiated thyroid cancer referred to our institution. Concerning disease progression at 1 year, the multivariate analysis showed that the presence of necrosis and the absence of radioactive iodine uptake were both prognostic indicators ($P=0.001$ for both). This suggests that radioactive iodine uptake, even if it does not significantly influence overall survival, can be predictive of a longer progression-free survival, even in patients with FDG uptake. To what extent this is linked to a therapeutic effect of radioactive iodine or to intrinsic characteristics of the tumor is still a matter of debate.

FDG uptake is correlated with clinical factors predicting a poor survival and the absence of response to $^{131}$I treatment, such as age over 45 years and the presence of necrosis (LiVolsi & Baloch 2002, Durante et al. 2006, Rivera et al. 2008). Necrosis and mitosis that are interrelated have already been shown to be highly prognostic in other endocrine cancers (La Rosa et al. 2009, Lau & Weiss 2009), and in recent studies, they were considered essential for the diagnosis of poorly differentiated thyroid carcinoma (Volante et al. 2004a,b, 2007). In this study, the architectural tumor pattern alone (papillary or follicular versus poorly differentiated) was not predictive of patient outcome or tumor aggressiveness. At histology, differentiated metastatic tumors frequently resulted in ‘complex tumors’ with frequent cellular atypias (70%), vascular invasion (71%), extension beyond the thyroid capsule (60%), necrosis (44%), and mitosis (52%). These characteristics may allow the definition of a subgroup of well-differentiated thyroid carcinomas, either with papillary or follicular histological features and with an aggressive behavior and in case of distant metastases these indicators will predict high-FDG uptake and poor outcome, with rapid progression and poor or no response to radiiodine treatment.

Immunohistochemical analysis and BRAF mutation analysis were performed only in some of the 80 patients because of the limited availability of paraffin blocks and of unfavorable material conditions for DNA extraction. Immunohistochemical analysis did not offer any adjunctive insights into prognosis or metabolic tumor behavior, and this may be related to changes in the histological and metabolic patterns between primary tumor and metastatic lesions (Hiltzik et al. 2006). Furthermore, the low frequency of BRAF mutation (10%) further suggests that tumors defined by their architectural pattern as well-differentiated were in fact very close to poorly differentiated on the basis of a high prevalence of necrosis, mitosis, and cellular atypias. This is in accordance with some recent reports of a low prevalence of BRAF mutation in advanced primary poorly differentiated thyroid cancers (Richarte-Filho et al. 2009, Volante et al. 2009).

In conclusion, this study confirms that FDG uptake is an independent prognostic factor in metastatic thyroid cancers and shows that it is associated with older age and specific histological tumor patterns (necrosis). All these parameters need to be studied in a larger cohort of patients in order to improve the prognostic classification of metastatic disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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